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ECTOMYCORRHIZAL INOCULUM POTENTIAL OF SOILS FROM FOREST RESTORATION SITES IN SOUTH VIETNAM

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Deforestation and conversion of land to agricultural use have resulted in the loss of vast areas of lowland rain forest in Southeast Asia and restoration of these forests through replanting programmes is now a priority. Members of the Dipterocarpaceae dominate the canopy in lowland rain forests, are among the most commercially important timber species and show much promise for reforestation work in the region (Salleh & Appanah 1994). Dipterocarpaceae also form symbiotic associations with ectomycorrhizal fungi, which are thought to be important in the successful establishment and growth of seedlings. Alexander *et al.* (1992) have shown that early infection of naturally regenerating dipterocarp seedlings depends on mycelial connections made by the ectomycorrhizal fungi associated with adjacent mature trees. It has also been shown that these mycelial networks are particularly sensitive to disturbance (Read & Birch 1988) and that forest clearance leads to rapid depletion of these sources of ectomycorrhizal inoculum in the soil (Brundrett 1991). In the absence of living dipterocarp roots and mycelial networks, spores of ectomycorrhizal fungi are the only potential source of inoculum for regenerating seedlings. The persistence of viable ectomycorrhizal spores in the soil following clearance is not known, but it is probable that inputs of fresh spores from fruitbodies produced in surrounding dipterocarp forest are the most important source of inoculum. The rate of ingress by these spores depends, to a large extent, on the proximity of the surrounding forest.

Successful establishment of dipterocarp seedlings at restoration sites will require that either soil at the site contains an adequate amount of ectomycorrhizal inoculum or that plants in the nursery are infected with the appropriate ectomycorrhizal fungi prior to outplanting. The capacity of a soil to form mycorrhizal associations (i.e. the number of infective mycorrhizal propagules in the soil) is known as the mycorrhizal inoculum potential (MIP) of the soil. At present, the best method of comparing the MIP of different soils is to grow non-mycorrhizal bioassay seedlings in intact soil cores over a short period and measure the extent of mycorrhizal development on the seedlings (Brundrett 1991). In this short study, we compared the MIP of soil taken from sites scheduled for replanting with soil from an undisturbed dipterocarp forest and a young dipterocarp plantation. The work formed part of a wider forest restoration project at Cat Tien National Park in South Vietnam.

Three sites were selected for sampling at Cat Tien National Park (107°25'E, 11°30'N). The sites were located near the Dong Nai River, in close proximity to each other, with each possessing similar alluvial soils subject to periodic flooding. Two of the sites (A and B) had been cleared of dipterocarp forest more than 30 years previously, converted to agricultural use in 1975 and were scheduled for replanting in 1997. In 1996, site A had been cultivated with sugar cane and site B with rice. The third site was an area (covering about 10 ha) of undisturbed dipterocarp forest dominated by mature trees of *Dipterocarpus intricatus*. The fourth site was a 13-y-old plantation of *Hopea odorata* located at Dinh Quán, about 30 km south of the sites at Cat Tien National Park. Like sites A and B at Cat Tien, this site had been cleared of dipterocarp forest more than 30 y previously. Since then it had been planted with *Tectona grandis* in 1975, before being cleared and planted with *H. odorata* in 1984.

On 13–14 May 1997, ten soil cores were collected from each of the four sites using a 5-cm diameter corer. The cores were immediately sealed in tightly fitting polythene bags and returned to the Centre for Seeds and Reforestation (CSR) Nursery at Long Thanh in Ho Chi Minh City. The following day each bag was opened and the undisturbed soil core planted with a pre-germinated seed of either *Dipterocarpus alatus* or *H. odorata* (both ectomycorrhizal dipterocarps). Seeds were germinated in Petri dishes on moistened filter paper and transferred within 1–2 days of germination. A split-plot design was adopted for the two plant species, the bags being laid out in two randomised blocks with soil from each site replicated five times within each block. Plants were harvested after five weeks and root systems carefully washed out, wrapped in tissue paper soaked in FAA (formalin-acetic-alcohol) and returned to the United Kingdom where root dry weight and numbers of mycorrhizal and non-mycorrhizal root tips were determined. Mycorrhizas were also separated into distinct morphological types using the methods of Ingleby *et al.* (1990) and tentative identifications made where possible by comparing with published descriptions (Jülich 1985, Agerer 1987–98, Ingleby *et al.* 1990, Agerer *et al.* 1996–98, Lee *et al.* 1997). Data were examined by two-way analysis of variance (ANOVA) using test soil and plant species as treatment factors. Before analysis, arcsine and log (n+1) transformations were performed on mycorrhizal infection percentages and root numbers respectively, and Bartlett's test (Sokal & Rohlf 1995) was used to ensure that sample variances were homogenous. Means were compared using Fisher's LSD test when the *F*-test from ANOVA was significant at $p < 0.05$.

After five weeks, root systems of *D. alatus* were much larger (root dry weight, $p < 0.001$; root tip number, $p < 0.001$) than those of *H. odorata* (Table 1). Although more ectomycorrhizal tips were found on seedlings of *D. alatus* than on *H. odorata*, the percentage of roots that were mycorrhizal was far greater on seedlings of *H. odorata* ($p < 0.001$). Ectomycorrhizal formation was observed on seedlings grown in soil from the undisturbed forest and plantation sites, but not in soil from either of the replanting sites (Table 1 and Figure 1). For both plant species, mycorrhizal infection was greatest on seedlings grown in the plantation soil and, for *H. odorata*, mycorrhizal infection on seedlings grown in the plantation soil was more than twice that of seedlings grown in soil from the undisturbed forest (Table 1). Greatest mycorrhizal diversity (4 types) was also found on seedlings of *H. odorata* grown in the plantation soil (Figure 1). The occurrence of mycorrhizal types on the seedlings suggested a degree of host-fungus specificity, as none of the four types found on *H. odorata* seedlings growing in the *H. odorata* plantation soil was found on *D. alatus* seedlings growing in the same soil. In contrast, the same type (Thelephoraceae) dominated seedlings of *H. odorata* and *D. alatus* grown in soil from the undisturbed forest.

Table 1. Root growth and mycorrhizal development of *Dipterocarpus alatus* and *Hopea odorata* seedlings grown in soil cores collected from Cat Tien National Park and a 13-y-old *H. odorata* plantation

Plant species	Parameter	Origin of soil				p value
		Cat Tien replanting site A	Cat Tien replanting site B	Cat Tien undisturbed forest	Dinh Quán *H. odorata* plantation	
D. alatus	Root dry wt. (mg)	165	161	141	206	0.420
	No. root tips	1473	2094	1072	2836	0.057
	No. mycorrhizal tips	0 c*	0 c	120 b	581 a	< 0.001
	% mycorrhizas	0 b	0 b	11.4 a	17.6 a	< 0.001
H. odorata	Root dry wt. (mg)	26.8	27.0	31.0	27.2	0.832
	No. root tips	113 b	329 a	315 a	443 a	0.011
	No. mycorrhizal tips	0 c	0 c	88 b	280 a	< 0.001
	% mycorrhizas	0 c	0 c	32.1 b	66.5 a	< 0.001

(Figures are mean values: n = 5).
* different letters after means within the same row indicate significant differences at $p < 0.05$ as determined by ANOVA and Fisher's LSD test.

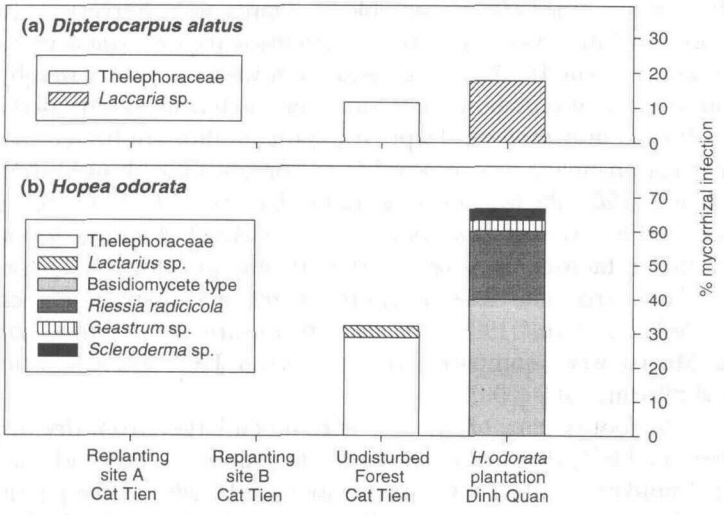

Figure 1. Occurrence and abundance of mycorrhizal types on (a) *Dipterocarpus alatus* and (b) *Hopea odorata* seedlings grown in soil cores collected from Cat Tien National Park and a 13-y-old *H. odorata* plantation

The results suggest that inherent differences between the plant species, such as host-fungus specificity, influenced mycorrhizal infection and diversity. In addition, mean seed weight of *D. alatus* (300 g/1000 seeds) was ten times greater than that of *H. odorata* (30 g/1000) and we hypothesise that the larger seed reserves of *D. alatus* promoted rapid root growth, which slowed the rate of mycorrhizal formation. These results support the views of Brundrett *et al.* (1996) who recommended the use of more than one plant species in bioassay tests.

The absence of any significant amount of ectomycorrhizal inoculum in the soils from the replanting sites may have been expected as these sites had been planted with agricultural crops for more than 20 years. In addition, the small areas of undisturbed forest adjacent to the sites might be considered as slightly degraded, possibly producing only a few ectomycorrhizal fruitbodies with concomitantly few spores being exported to surrounding areas. The soils were also subject to periodic flooding which may have affected the survival of any spores present. These factors may also explain the low infection found on bioassay plants grown in soil from the undisturbed forest. Another possible explanation is that many of the fungi present in the soil were unable to infect the seedlings after removal from the forest. Successions of ectomycorrhizal fungi are known to occur as forest communities age (Mason *et al.* 1983), and it is thought that fungi which dominate in mature forests are only able to infect seedling roots via mycelia connected to mycorrhizas of the mature trees (Fleming 1984). Therefore many of the fungi present in soil from the undisturbed forest may have been unable to infect the bioassay seedlings. In contrast, soil from the *H. odorata* plantation may have possessed a range of pioneering fungi, which were able to infect the bioassay seedlings via germinating spores or fragmented mycelium.

The absence of ectomycorrhizal infection in soils from the replanting sites confirmed that ingress by fungi in the nursery had not occurred and that the mycorrhizas found on the seedlings originated from the test soils. The five-week growth period employed for this bioassay test therefore provided a reliable and rapid indication of potential mycorrhizal deficiencies at the sites scheduled for forest restoration. Adequate mycorrhizal development on nursery plants is recommended if plants are to survive at these replanting sites. The study also indicated that ectomycorrhizal fungi which dominate in young plantations are more likely to successfully colonise seedlings in the nursery and that such fungi should be used when nursery inoculation of dipterocarps is deemed necessary.

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